Supplementary Information

Ribosomal RNA 2'-O-methylations regulate translation by impacting ribosome dynamics

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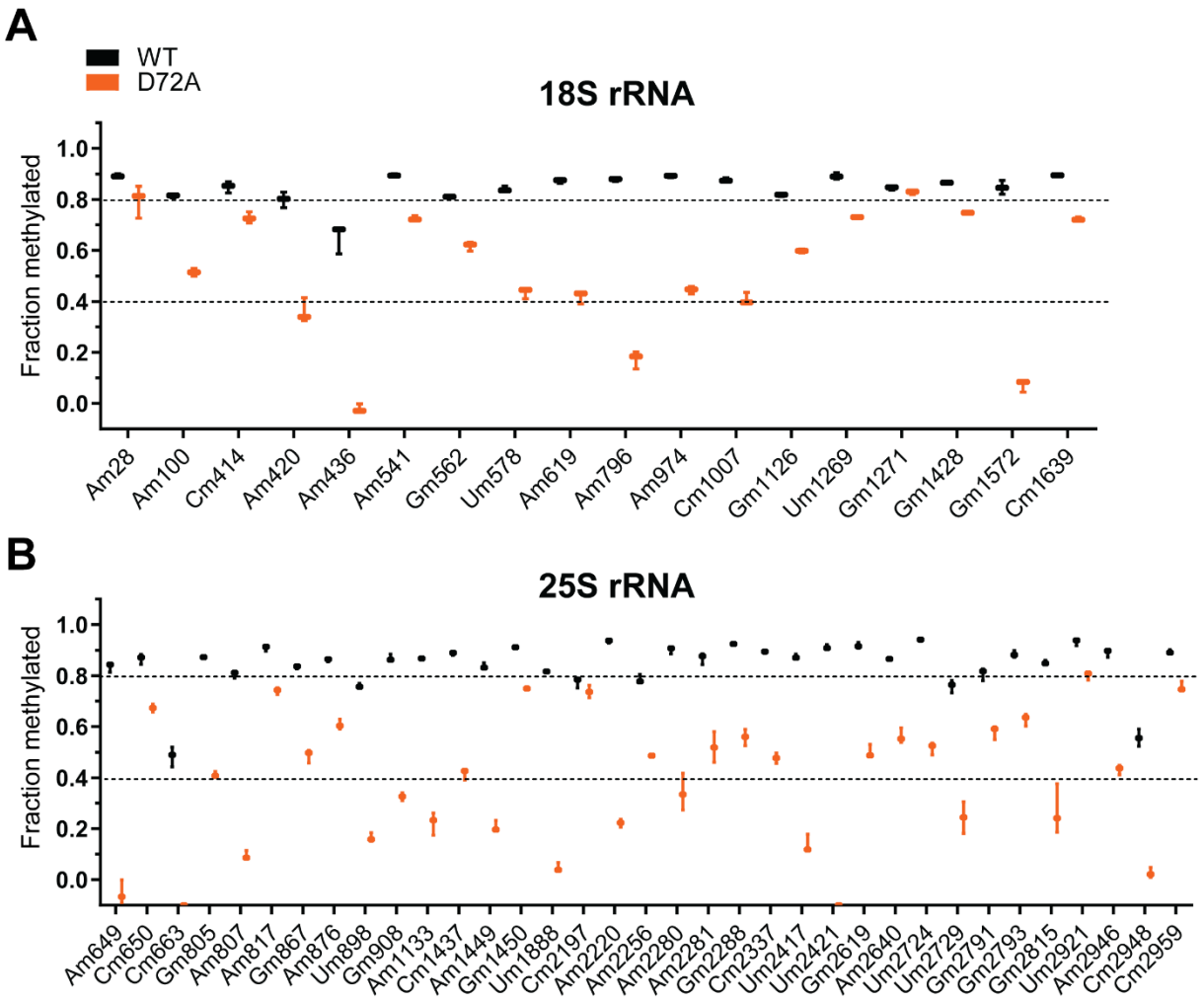

Figure S1. Comparison of the 2'-O-methylation sites. MethScores at each modified position of 18S **(A)** and 25S rRNA **(B)** are compared between wild-type control (WT) and *bcd1-D72A* (D72A) cells. RiboMethSeq was performed on three biological replicates. Box edges represent the interquartile range, midlines indicate the median, and lower and upper whiskers extend to the min and max, respectively.

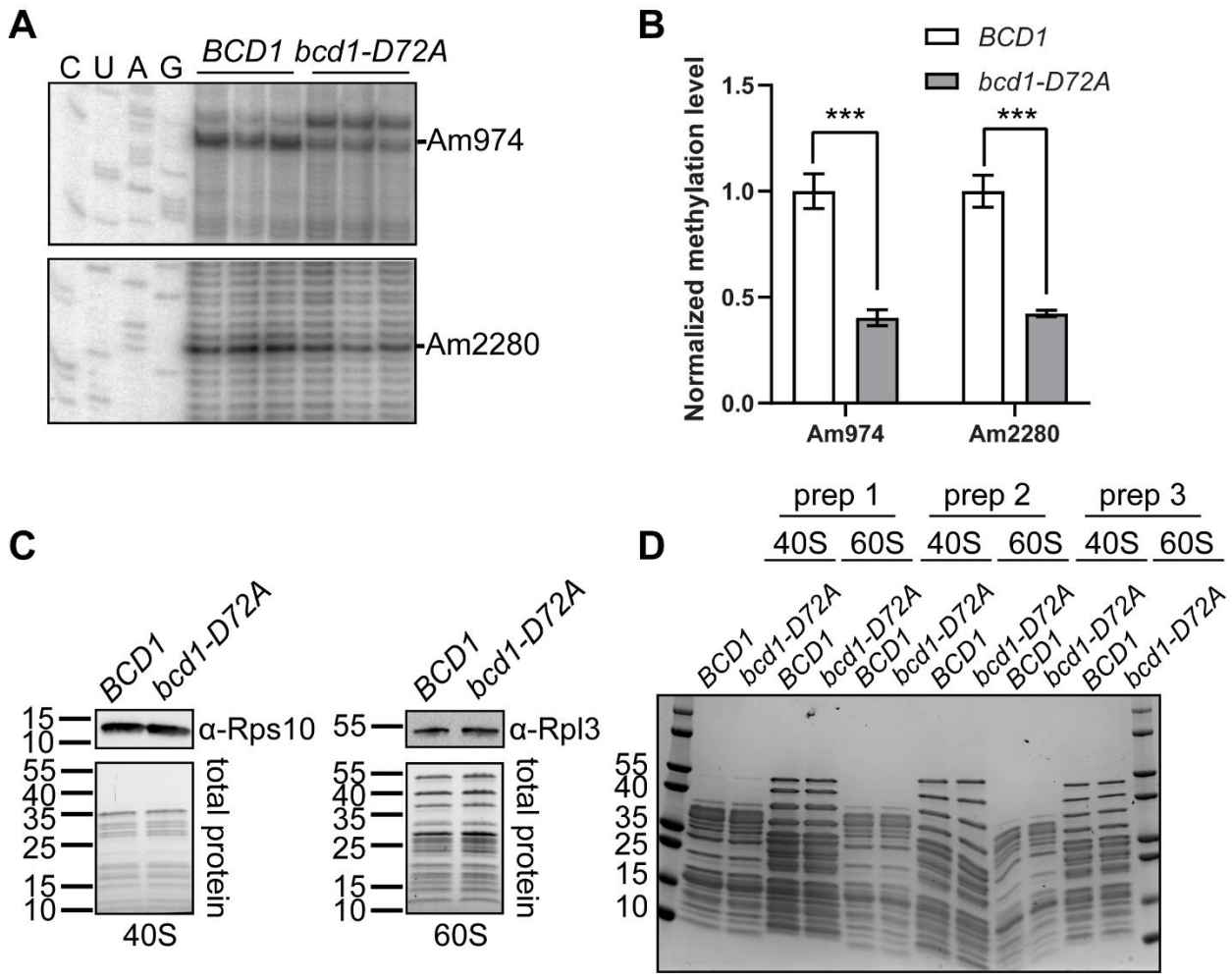

Figure S2. Comparing the modification and composition of mature ribosomes from *BCD1* and *bcd1-D72A* cells. (**A**) Reverse transcription at low concentration of dNTP combined with sequencing gel analysis was used to determine the methylation levels at a methylation site in 18S rRNA (Am947, upper panel) and 25S rRNA (Am2280, lower panel) of isolated mature ribosomes. Ribosomes isolated from three biological replicates were analyzed. (**B**) Quantification of the data shown in A. The intensity of bands at the reverse transcription stops were normalized to all band intensities below the stop signal. Both SSU A974 and LSU A2280 are less than 50% modified relative to the wild-type control, similar to their relative methylation levels as quantified by RiboMethSeq. Graph bars represent the mean and standard deviations from three biological replicates. Significance was analyzed using a t-test. ***p ≤ 0.001. (**C**) 40S and 60S ribosomes purified from *BCD1* and *bcd1-D72A* cells have the same composition as judged by Western blots against two ribosomal proteins. Top panels: Western blot against Rps10 (40S) or Rpl3 (60S); bottom panels: total ribosomal proteins. Rps10 antibody was a gift from K. Karbstein and Rpl3 antibody was obtained from the Developmental Studies Hybridoma Bank. (**D**) 40S and 60S ribosomes purified from *BCD1* and *bcd1-D72A* cells have the same composition as judged by SDS-PAGE. Three independent ribosome preps are shown.

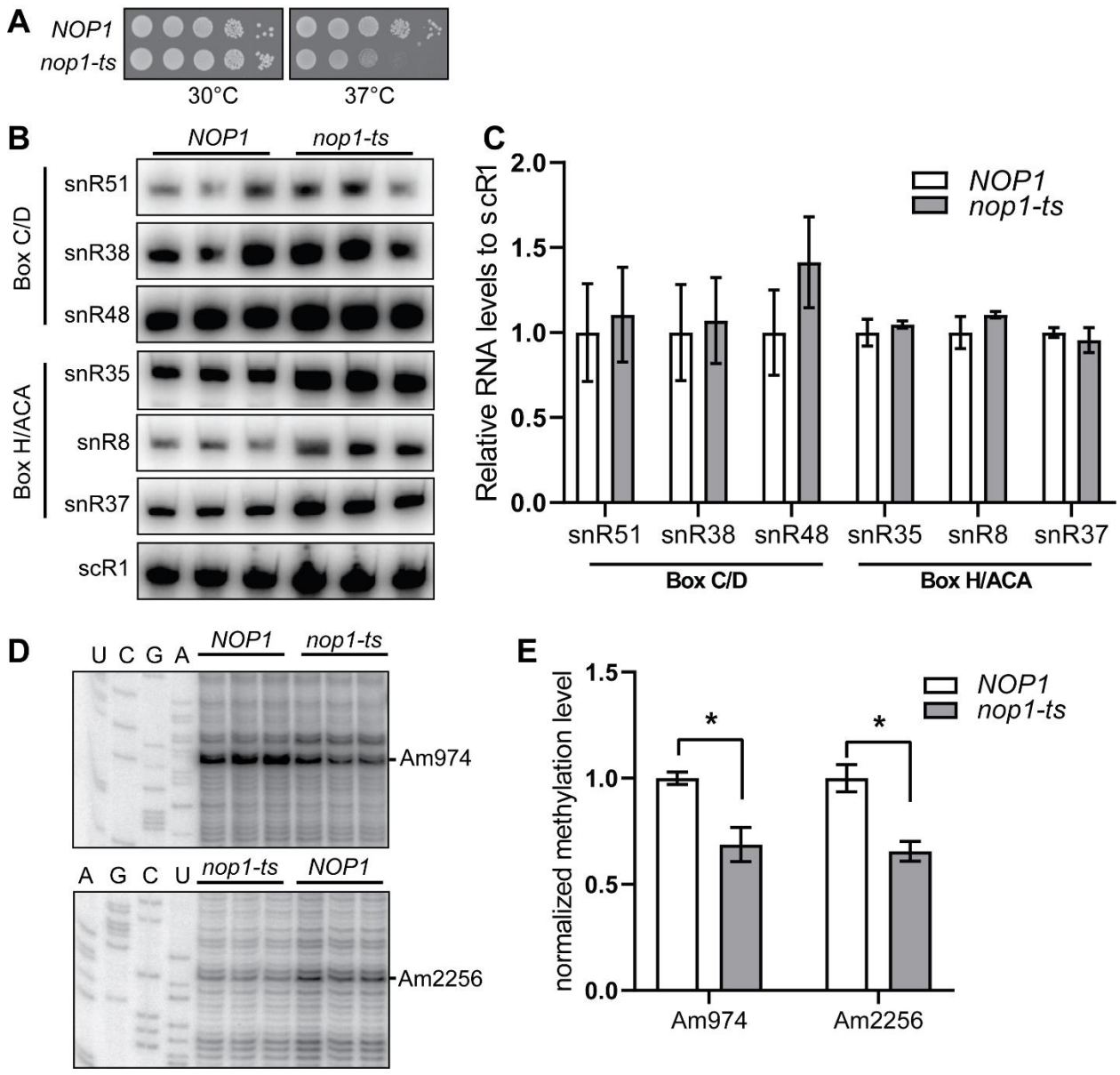

Figure S3. *nop1-ts* affects the rRNA 2'-O methylation but not the snoRNA levels. (A) *NOP1* and *nop1-ts* cells were serially diluted and spotted on YPD plates and incubated at 30°C or 37°C for two days. **(B)** Northern blotting analyses of steady-state expression levels of box C/D and box H/ACA snoRNAs in *NOP1* and *nop1-ts* cells. **(C)** Quantification of data shown in B as normalized to scR1. Graph bars represent the mean and S.D. from three biological replicates. There was no significant difference in the snoRNA levels between *NOP1* and *nop1-ts* cells as determined using a t test. **(D)** Reverse transcription at low concentration of dNTP combined with sequencing gel analysis was used to determine the methylation levels at a methylation site in 18S rRNA (Am947) and 25S rRNA (Am2256). **(E)** Quantification of the data in D. Graph bars represent the mean and standard deviations from three biological replicates. Significance was analyzed using a t-test. *p ≤ 0.05.

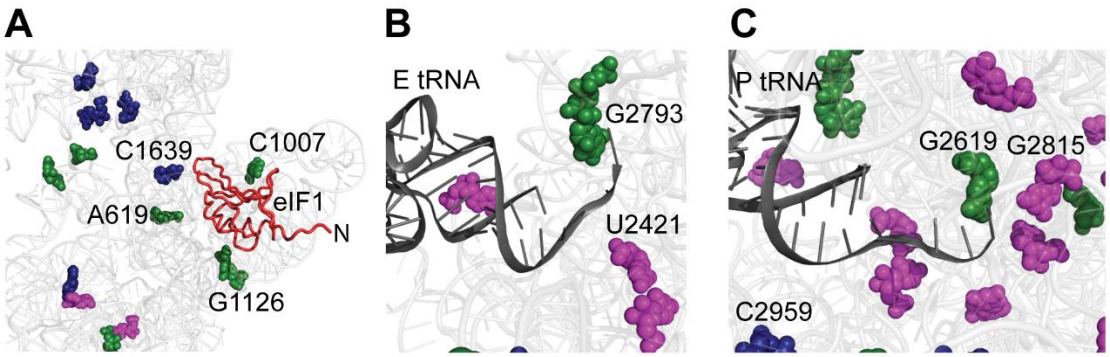

Figure S4. Position of rRNA 2'-O-methylations relative to eIf1 and the E- and P-site tRNAs. (**A**) eIF1 binding site (PDB: 6gsm). (**B**) The E-site tRNA (**C**) the P-site tRNA (PDB: 3j78). Methylation sites follow the same color coding as in Figure 1. The rRNA is in light gray, the eIF1 in red and the tRNA in dark gray.

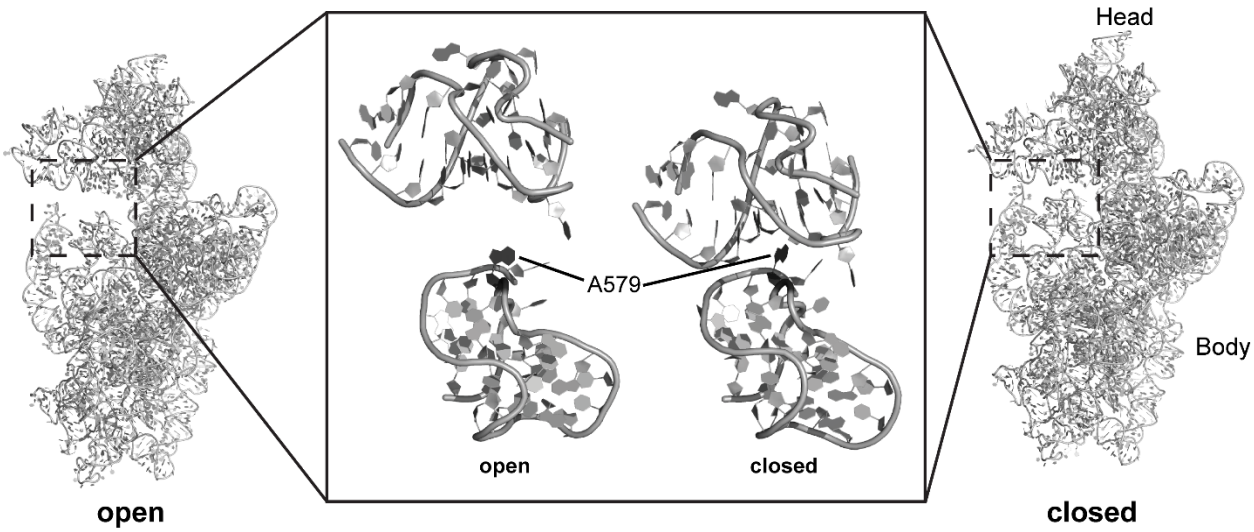

Figure S5. Comparing the position of SSU-A579 in the open and closed conformations. The left and right structures are the 18S rRNA in the open (PDB: 3jaq) and closed (PDB: 3jap) conformations, respectively. The position of the mRNA latch is boxed in both structures and zoomed in in the middle panel.

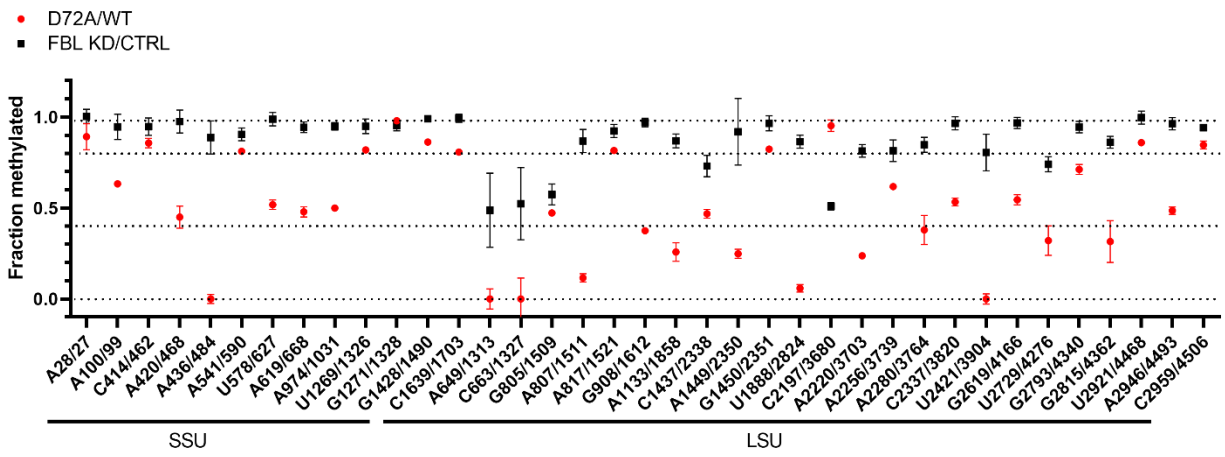

Figure S6. Comparison of the changes in rRNA 2'-O-methylation levels of the sites conserved between yeast and human. The red squares show mean methylation scores at each indicated site in *bcd1-D72A* cells relative to control wild-type yeast. The black squares show mean methylation scores from HEK cells after fibrillarin knockdown relative to control (1). Error bars represent standard deviations.

Table S1: Lack of correlation between the snoRNA and 2'-O methylation levels.
Comparison of the snoRNA levels corresponding to the most stable 2'-O-methylation sites (mean MethScore > 0.8) in 18S and 25S rRNA.

	2'-O-methylation site		Box C/D snoRNA level	
18S	A28	0.89	snR47	0.12
	C414	0.86	snR128	0.30
	A541	0.81	snR41	0.24
	U1269	0.82	snR65	0.20
	G1271	0.98	snR40	0.34
	G1428	0.86	snR56	0.13
	C1639	0.81	snR70	0.17
25S	A817	0.82	snR60	0.17
	C1450	0.82	snR24	0.32
	C2197	0.95	snR76	0.75
	U2921	0.86	snR52	0.09
	C2959	0.85	snR73	0.18

Table S2: **Plasmids used in this work**

Plasmid	Description	Backbone	Reference
pDH412	*RPS3*	pRS315	(2)
pDH425	*rps3*-R116D	pRS315	(2)
pDH482	*rps3*-R117D	pRS315	(2)
HG3248	*RPL3*	pRS313	This study
HG3254	*rpl3*-W255C	pRS313	This study
HG3255	*rpl3*-H256A	pRS313	This study
HG3209	*SUI1*+UTRs	pRS425	This study
HG3210	*TIF11*+UTRs	pRS425	This study
HG3293	*EFT2*	pRS415	This study
pSRT209	Dicistronic reporter with a wild-type CrPV IGR IRES and a ΔAUG for the luciferase codon that reduces background from cryptic promoters	pRS425	(3)
pSRT210	Identical to pSRT209 except for the 2 nucleotides change in the IRES to disrupt PKI	pRS425	(3)
pDB722	PGK-Readthrough	pYEplac19	(4)
pDB723	PGK- Stop	pYEplac19	(4)
pDB868	PGK- Mis3coding Firefly H245R	pYEplac19	(5)
pJD375	ADH-0-Frame Control	pRS416	(6)
pJD376	ADH L-A (-1) Frameshift	pRS416	(6)
pJD377	ADH Ty1 (+1) Frameshift	pRS416	(6)
pRaugFuug	RaugFuug ADH/GPD start	pRS416	(7)
pRaugFaug	RaugFaug ADH/GPD start	pRS416	(7)
HG187	6xHis-TEV-eIF1	pET23a	This study

Table S3: **Yeast strains used in this work**

Strain name	Description	Genotype	Reference
yHG000	BY4741	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	GE Dharmacon
yHG458	*bcd1-D72A*	*BY4741; bcd1-D72A*	(8)
yHG520	*ΔRPL3+ rpl3-W255C*	*BY4741; RPL3::NAT*	This work
yHG521	*ΔRPL3+ rpl3-H256A*	*BY4741; RPL3::NAT*	This work
yHG561	*GAL:RPS3*	*BY4741; GAL:RPS3 (KAN)*	This work
yHG562	*bcd1-D72A; GAL:RPS3*	*BY4741; bcd1-D72A; GAL:RPS3 (KAN)*	This work

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